JPRS-JST-90-005
17 JANUARY 1990

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BIO-FUNCTIONAL MATERIALS

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SCIENCE & TECHNOLOGY

JAPAN

BIO-FUNCTIONAL MATERIALS

[Selected abstracts on the design, structure, and functions of bio-functional materials; "priority areas of research" sponsored by the Ministry of Education, Science and Culture]

43068010 Tokyo MONBUSHO JUTEN RYOIKI KENKYU SEIKA HOKOKUSHO in English Mar 89 pp 1-760

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Synthesis of Peptide Materials Having Biospecific Recognition Functions

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1. Introduction

In the present investigation, the response of cells to biosignal molecules, in particular, cell-growth factors, which are immobilized to polymer film, is investigated. The growth factor-immobilized polymer film may well function as cell-culture materials. It is the feature of the present investigation that nonbiodegradable and nonhydrogel polymer matrix is used for the immobilization.

In the present investigation, the control of cell adhesion to polymer film, to which cell-adhesion peptides Arg-Gly-Asp-Ser (RGDS) are immobilized, is also investigated. The cell adhesion peptide-immobilized polymer film may lead to the development of biocompatible materials.

2. Results and Discussion

2.1 Control of cell growth on the cell growth factor-immobilized polymer film

We have reported that the cell growth is accelerated on insulin- or transferrin-immobilized poly(ethylene terephthalate) or poly(methyl methacrylate) film and that the acceleration effect is stronger than that in the case of free insulin or transferrin addition. In the present investigation, the effect of a simultaneous immobilization of cell growth factors of different kinds as well as the immobilization with intervening spacer chains was investigated. However, the large cooperative effect of the simultaneous immobilization was not observed. On the other hand, the effect of the spacer chain was observed, which indicates that the presence of the spacer chain amplified the stimulation to the cell by enhancing the diffusion of the growth factor/receptor complex in the cell membrane and by accelerating the patch formation. The cell growth factor-immobilized polymer film can be used for cell culture without serum.

2.2 Control of cell adhesion on the cell adhesion peptide-immobilized polymer film

We have reported that the RGDS-immobilized film adhered a large number of fibroblast cells which is comparable to that adhering to the fibronectin-immobilized film. In the present investigation, it was shown that the RGDS-immobilized film is more stable against the treatment with ethanol, heating and pH change than the fibronectin-immobilized film.

Molecular Structural Approach to the Design of the Functions of Immunoglobulins

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Molecular structural analyses of immunoglobulin molecules will be made for providing them with a variety of new biological functions. Immunoglobulins selectively labeled with stable isotopes including ^{2}H and ^{13}C are being used along with photo CIDNP method for the NMR analyses.

Anti-dansyl switch variant monoclonal antibodies have been labeled with methionine-1-^{13}C. Site-specific assignments of some of the carbonyl carbons have been made using a double labeling technique developed by Kainosho and coworkers. We have also made domain specific assignments of ^{1}H NMR signals of tyrosines. It has been concluded that the structure of the antigen binding site is significantly affected by the presence or absence of the constant domains C_L and C_H1. This result strongly suggests that the presence of the constant domains has seriously be taken into account when the antigen binding domains are to be mimicked using solely the V_L and V_H domains.

Cell Separation by Polymeric Materilas with Controlled Micro-Structure

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1. Introduction

An increasing demand for pure and vital cell populations greatly facilitates the development of novel methods for cell separation. Detailed study on cellular adhesion to polymer surfaces with controlled super structure contributes to the development of novel materials for cell separation as well as to the elucidation of mechanisms involved in cellular recognition of polymer surface structures. An important facet of this study is to quantitatively evaluate the cellular adhesion to materials with a particular microdomain structure. For this purpose, a new method named hybrid field-flow fractionation (FFF)/ adhesion chromatography was developed. Adhesion strength of B and T lymphocytes on poly(2-hydroxyethyl methacrylate)/polyamine graft copolymer (HA), a polymer showing highly selective affinity toward B lymphocytes, was evaluated under a controlled shear stress.

2. Results and Discussion

Thin ribbon-like chamber with an open-flow channel was prepared by combining two glass plates with 250 um Teflon spacer. Inner-bottom surface of the chamber was coated with HA copolymer. Lymphocytes were introduced into the chamber, settled there for a given time period, and then, recovered from the chamber by a stepwisely increasing flow. Adhesion strength of lymphocytes on HA-coated surface was strongly affected by a surrounding temperature, and increased with a rise in temperature from 4°C to 23°C, reflecting the sol-gel transition of plasma membrane of lymphocytes. Lymphocyte recovery from the chamber was also affected by the settling time as well as by flow rate. At 4°C with 5 min settling, B lymphocyte was found to adhere 5 times more strongly on HA surface than T lymphocyte. From a partical point of view, almost quantitative separation of B and T lymphocytes was achieved by this new methodology.

Molecular Design of Polymeirc Materials with Specific Cellular Recognition

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1. Introduction

Materials with specific recognition toward a particular cell population has a great feasibility as column adsorbent for cellular adsorption chromatography. Through our systematic study on cell-materials interactions, we have found that poly(2-hydroxyethyl methacrylate)/polyamine graft copolymer (HA) selectively adsorbs B lymphocytes out of a mixture of B and T lymphocytes collected from rat mesenteric lymphnode. Based on this result, we have designed in this study a partially quaternized HA copolymer (HQA) to achieve more sensitive resolution of lymphocyte subpopulations, including T lymphocyte subsets, and to demonstrate that our strategy to resolve lymphocyte subpopulations through controlled ionic interaction is valid for lymphocytes collected from animal species other than rat.

2. Results and Discussion

Adsorbent was prepared by coating HQA on glass beads. Lymphocyte suspension prepared from rat mesenteric lymphnode was passed through a column packed with adsorbent thus prepared. As is the case with HA, HQA showed high adsorbability toward B lymphocytes. Further, there existed an HQA-adsorbed fraction in T lymphocyte population. Immuno-fluorescence assay using FITC-labeled monoclonal antibody revealed that the HQA-adsorbed fraction of T lymphocyte mainly consists of helper T lymphocyte. Selective retention of helper T lymphocyte became much significant by lowering pH of the suspension from 7.4 to 6.8. No such resolution was observed for HA column, indicating a crucial role of the quaternized ammonium groups in a resolution of T lymphocyte subsets by HQA. Another unique feature of HQA is its high capability of separating murine as well as rat lymphocyte subpopulations, suggesting a wide applicability of cationic graft copolymer as adsorbent for cell separation.

Synthesis of Sulfated Polysaccharide Having High Anti-AIDS-Virus Activity and Limited Anticoagulant Activity

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1. Introduction

We reported that sulfated polysaccharides have high anticoagulant activity. Recently, we also found that sulfated polysaccharides protected HIV (human immunodeficiency virus = AIDS virus) induced cytopathic effects (CPE). The purpose of this project is synthesis of sulfated polysaccharide which strongly inhibits the HIV-induced CPE and weakly interacts with blood coagulation factors. Sulfated branched polysaccharides were synthesized.

2. Results and Discussion

Anti-HIV effects of lentinan sulfate were investigated by using an HTLV-I-carring cell line, MT-4, in vitro. Lentinan, a fungal branched (1→3)-β-D-glucan, was sulfated to different degrees with two kinds of procedures using piperidine N-sulfonic acid in dimethylsulfoxide or chlorosulfonic acid in pyridine. Lentinan sulfate with sulfur content of more than 14% completely prevent HIV-induced CPE in concentrations of more than 3.3 μg/ml. However, the low-substituted lentinan sulfate did not prevent HIV-induced CPE in any concentrations tested. When the counter-cation was 50% sodium ion and 50% pyridinium ion, the inhibition capacity was low. Lentinan sulfate with sulfur content of 16% and $\bar{M}_n$ of 1.8×10^4 showed relatively low anticoagulant activity (21 unit/mg), while lentinan sulfate with sulfur content of 14% and $\bar{M}_n$ of 4.8×10^4 showed high anticoagulant activity (54 unit/mg).

Ring-opening polymerization of 1,6-anhydro-2,4-di-O-benzyl-3-O-t-butyldimethylsilyl-β-D-glucopyranose and selective ring-opening copolymerization of 1,4-anhydro-2,3-di-O-t-butyldimethylsilyl-α-D-ribopyranose with 1,4-anhydro-2,3-di-O-benzyl-α-D-ribopyranose gave stereoregular polysaccharides. Obtained polysaccharides were selectively deprotected and glycosylated with sugar orthoesters. Synthetic branched polysaccharides were sulfated with piperidine N-sulfonic acid in DMSO to give sulfated branched polysaccharides, which had anti-HIV activity.

Design and Preparation of Materials Controlling Cell Attachment and Growth

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1. Introduction

There is increasing research and development in biocompatible polymers. However, a systematic study of the effect of polymer structure on biocompatibility is lacking. A study of cell behaviors on the polymer surface will provide the useful basis for tailoring biofunctional polymers. In the past, we prepared ethylene / vinyl alcohol copolymers derivatized with various molecules and studied cell behaviors on their surfaces. In this year, polyurethanes have been selected as potential materials for biomedical applications. In addition to the study on cell behaviors on polyurethanes, we initiated study on in vivo stability of polyurethanes, because polyurethanes should be stable if the polymers are going to be used for long-term practice.

2. Results and Discussion

Polyurethane having hydroxyl group was prepared from diphenylmethane diisocyanate (MDI), poly(tetramethylene glycol)(PTMG) and diaminoisopropanol and the hydroxyl group was further modified with perfluorooctanoyl group. Ten fluorine-containing polyurethanes were also synthesized from MDI, PTMG, N,N-bis(2-hydroxyethyl)perfluorooctanesulfonamide and 1,4-butanediol or ethylenediamine. Ca.9.22 epithelial cells were incubated on the films of polyurethanes synthesized, and cell attachment and cell growth were measured.

Introduction of perfluorooctanoyl moiety to polyurethane effected increase in water contact angle, but had no significant effect on cell attachment and growth. The polyurethanes containing perfluorooctanesulfonamide unit showed different cell behavior between the type of polyurethanes. The polyurethanes chain-extended with the diamine showed considerably lower cell attachment than those with the diol.

The retrieved polyurethane samples, which had been implanted in rat for upto 28 months, were subjected to ATR-IR analysis, GPC analysis and tensile property testing. Tensile strength did not change so significantly during the period. However, GPC and ATR-IR data clearly showed gradual degradation of urethane bonds.

Recognition and separation of antigen by antibody immobilized on bacterial magnetite.

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1. Introduction

Magnetotactic bacteria contained about 10 to 20 magnetic particles. These magnetic particles consisted of magnetite (Fe_3O_4). They were 500-1000Å in size and covered with organic thin films. These bacterial magnetites were applied to the immobilization of FITC conjugated anti-mouse IgG for the measurement of mouse IgG.

2. Results and Discussion

Bacterial magnetites were separated from magnetotactic bacteria with magnet after the ultrasonication. Bacterial magnetites treated with chloroform-methanol were not covered with magnetosome membrane. Magnetosome membrane was mainly consisted of lipid. Thin layer chromatography analysis showed that these lipids consisted of phospholipids and glycolipid. One of the phospholipids was identified as phosphatidyl ethanolamine. Four saturated fatty acids ($C_{12:0}$, $C_{14:0}$, $C_{15:0}$, $C_{16:0}$) and two unsaturated fatty acids ($C_{16:1}$, $C_{18:1}$) were identified. Palmitoleic acid and oleic acid accounted for 90% of the total fatty acids. Moreover, the measurements of mouse IgG concentration were carried out by using FITC conjugated anti-mouse IgG immobilized on bacterial magnetites and a fluorescence spectrophotometer. The quantities of antibody coupling with bacterial magnetites were 263 μg/mg particles, while artificial magnetites were 68 μg/mg particles. The reaction between the antigen and the antibody immobilized on bacterial magnetites was enhanced by magnetic field. Mouse IgG concentration could be measured in the range of 0.5-100 ng/ml.

Structure and Properties of New Bacterial Polyesters

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1. Introduction

An optically active copolyester of 3-hydroxybutyrate (3HB) and 3-hydroxyvalerate (3HV) has been produced from pentanoic and butyric acids by Alcaligenes eutrophus. The copolyester compositions varied from 0 to 95 mol% 3HV units, depending on the composition of the carbon sources. In addition, we have found that a new type of random copolyester of 3HB and 4-hydroxybutyrate (4HB) is produced from 4-hydroxybutyric and butyric acids by A. eutrophus. The present research aims at developing new bacterial polyesters with biodegradable and biocompatible functions.

2. Results and Discussion

The melting temperature of P(3HB-co-4HB) decreased from 180 to 150°C as the 4HB fraction increased from 0 to 49 mol%. The X-ray crystallinity of P(3HB-co-4HB) film decreased from 60 to 10% with an increase in the 4HB fraction. The film became soft with the 4HB fraction, and the strain increased from 5 to 444% at 16 mol% 4HB. The true tensile strength of P(3HB-co-16%4HB) was over 100 MPa. The copolyesters over 40 mol% 4HB fraction exhibited the mechanical properties of an elastic rubber.

The biodegradation of copolyester films was studied in soil and activated sludge. The rate of biodegradation was strongly dependent upon the molecular structure and composition of copolyesters. It has been found that the rate of biodegradation is enhanced by the presence of 4HB units.

Preparation and Evaluation of Protein-Separation Membranes Modeled after Glomerular Capillary Wall

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1. Introduction

The glomerular capillary wall markedly restricts the transmural passage of plasma proteins and other macromolecules, while at the same time it permits high rate of fluid filtration. However, no report had been published on the synthetic charged ultrafiltration membrane for the permeation and separation of proteins, until we reported a preliminary result. In this study, positively charged membranes were used to examine the permeation of γ -globulin and the separation from albumin, the other plasma protein.

2. Results and Discussion

Polyacrylonitrile graft polymer with side chains of dimethylaminoethyl methacrylate was photochemically synthesized. The positively charged ultrafiltration membranes modeled after glomerular capillary wall were made by casting the graft polymer. The cut-off molecular-size of about 500 Å was estimated from the measurement of permeability for dextrans having various molecular weights. The permeation of albumin and γ -globulin through the membranes was measured by using a buffered saline solution containing both proteins. It was found that the permeation of albumin was greater than that of γ -globulin in the solution at pH higher than the isoelectric point of albumin. Taking account the molecular size of both proteins, this observation indicates that the separation of the proteins was due to the interaction between the charge of the proteins and that of the membrane.

Interfacial Chemical Study on Preparation of Biofunctional Polymer Latices and their Dispersion Stability

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1. Introduction

Rembaum et al. reported on the fixation of antibodies onto polymer latex particles by chemical bonding. They applied latex particles having functional groups such as carboxyl groups. Their results suggest that hydrophilic functional polymer latex is advantageous as carriers of antibodies. From these points of view, in this work, we have prepared styrene/acrylamide/acrylic acid copolymer (P(St/AAm/AA)) and styrene/2-hydroxyethyl methacrylate/acrylic acid copolymer (P(St/HEMA/AA)) latices as hydrophilic functional polymer latex and investigated their surface characteristics and dispersion stability. In addition, the flocculation behavior of their latices coated in advance with bovine serum albumin (BSA) was investigated as a function of surface coverage of BSA. The influence of the content of hydrophilic monomer, viz, AAm and HEMA is considered.

2. Results and Discussion

P(St/AAm/AA) and P(St/HEMA/AA) latices with different content of AAm and HEMA were prepared without emulsifier using potassium persulfate as initiator. The surface characteristics and the colloidal stability of their latices were investigated as a function of the amount of AAm and HEMA. The surface charge density of the latex particles steeply and continuously increases with increasing pH. The colloidal stabilities for NaCl concentration were remarkably enhanced with increasing both pH and the content of AAm and HEMA. These results suggest that the contraction and expansion of water-soluble polymer layer surrounding the particles play an important role in the surface properties and colloidal stability.

The flocculation behavior of P(St/AAm/AA) and P(St/HEMA/AA) latices coated in advance with BSA was investigated as a function of surface coverage ( ) of BSA and content of hydrophilic monomer. The flocculation behavior of BSA covered latices depends on that of uncoated latices, and the critical flocculation concentrations (cfc) of BSA coverd latices increase with increasing content of hydrophilic monomer, viz, AAm and HEMA. These results suggest that the colloidal stability of polymer latices in albumin solution such as blood is enhanced by copolymerizing hydrophilic monomer.

AN ATTEMPT TO DEVELOP THE DETECTION AND SEPARATION METHODS BY THE USE OF PROTEINS WHICH LOST THE CATALYTIC FUNCTION

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1. Introduction

The methods for the selective detection as well as the fine separation of the intended substances are necessary for the development of much more elaborate and highly effective bio-reactor system. We attempt to develop newly detecting and separating methods by the use of enzyme molecules, of which the catalytic activities are lost by chemical modifications or missense mutations.

2. Results and Discussion

To achieve our purpose, in the present work, we have coupled alkaline phosphatase to purous glass by means of polyethylene glycol (PEG) (mean M.W., 4000) spacer, and we have compared the amount and activity of the PEG-bound enzyme to enzyme immobilized by conventional, short urea linkage. Outline of attachment procedures is shown by equations 1-3 below.

$$\text{glass-SiOH} + (\text{EtO})_3\text{Si}(\text{CH}_2)_3\text{-R} \longrightarrow \text{glass-}(\text{CH}_2)_3\text{-R} \qquad \text{Eq. 1}$$

$$\text{R= -NH}_2 \text{ or -N=C=O}$$

$$\text{glass-}(\text{CH}_2)_3\text{-NH}_2 + \text{M-PEG-M} \longrightarrow \text{glass-}(\text{CH}_2)_3\text{-NH-D-PEG-M}$$

$$\xrightarrow{+\ \text{Enz-NH}_2} \text{glass-}(\text{CH}_2)_3\text{-NH-D-PEG-D-NH-Enz} \qquad \text{Eq. 2}$$

M=mono- and D=disubstituted s-triazine, Enz-NH_2= enzyme

$$\text{glass-}(\text{CH}_2)_3\text{-N=C=O} + \text{Enz-NH}_2 \longrightarrow \text{glass-}(\text{CH}_2)_3\text{-NHCONH-Enz} \qquad \text{Eq. 3}$$

Roughly comparable amounts (on a molar basis) of activated PEG and isocyanate are attached to the glass, with somewhat more isocyanate than PEG. The size of the PEG molecule as compared to the isocyanate, and the fact that PEG attached in two steps, likely accounts for the greater sufrace coverage by isocyanate.

As could be expected on the basis of higher loading of enzyme, the isocyanate route then gives a greater total activity, although not all of the advantage of loading excess is preserved; specifically, 33 times as much enzyme is immobilized via the isocyanate route, but the route gives only 10 times the total enzyme activity. Interestingly, enzyme immobilized via the PEG linker is almost as active as native enzyme, while that coupled to the surface by the urea linkage has lost 74% of its activity, suggesting that the enzyme immobilized by the short urea linkage could be expected to be conformationally restricted by its close proximity to the rigid surface.

Preparation of Hybrid Microspheres and Their Applications as Bio-Separators

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There are some special proteins playing an essential role in transcription of gene. Separation and purification of these proteins are very important not only from a scientific view but also from a technological one. In this study DNA chains having a specific base sequence were bound to latex particles and a kind of above-mentioned proteins was purified from the mixture through the affinity of the protein and on-surface DNA chains.

Latex particles employed as the carrier of DNA chains must be free from non-specific adsorption and interactions. They must have functional groups suitable for the chemical binding with DNA chains.

Glycidyl methacrylate (GMA) was expected to give such properties to latex particles. GMA alone or GMA and styrene (St) were polymerized in soap-free aqueous media by using potassium persulfate as an initiator. The resulting latex particles having submicron size were hydrolyzed to convert the ethoxy groups to glycol groups. Poly-GMA particles excellently prevented non-specific adsorption, but St-GMA copolymer particles did not.

Complementary 5'-phosphorylated oligonucleotides, which are specifically recognized by a trinscription factor, E4TF1, were polymerized to ca. 300 base pair and attached to poly-GMA particles by using BrCN under the best condition searched with respect to the reaction temperature, pH, the concentration of DNA chains, etc.

The DNA-carrying latex particles were added to a mixture of proteins and the supernatant was analyzed by SDS-page and gel shift assay. Separation of latex particles from the medium was easy because of the high specific gravity of particles. The E4TF1 protein was found to be adsorbed from 0.1 M KCl Tris buffer and desorbed with 0.5 M KCl Tris buffer. Thus, E4TF1 was successfully separated and purified from the mixture of proteins in batch process. Repetitive application of the above process in the presence of poly[dI-dC] resulted in excellent purification of E4TF1.

I greatfully acknowledge Dr. Hiroshi Handa, Tokyo University, for his cooperation in this work.

Modifications of Polysaccharides for Enhancing Abilities of Separation and Chiral Recognition

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1. Introduction

Advanced separation procedures are becoming increasingly important recently in various fields. Polysaccharides and the derivatives are considered to have advantages in many respects in producing novel separating materials owing to their structural characteristics. We have undertaken a systematic investigation of establishing efficient and versatile chemical modification modes and thereby developing novel separating materials based on polysaccharides, primarily chitin.

2. Results and Discussion

(1) Chitin/Polypeptide Hybrid Materials

A procedure for efficient graft copolymerization of γ-methyl L-glutamate NCA has been established. Properties of the resulting derivatives were dependent on the side chain length. They were evaluated as dialysis membranes, and the permeability was controlled effectively by the side chains, especially by the α-helix formation.

Chitin/polyalanine hybrid materials were also prepared, and an active site of NADH was attached to the polyalanine side chains with the expectation of realizing much improved asymmetric recognition in the reduction of ketones.

(2) Iodo-Chitins and Graft Copolymerization

Preparation of iodo-chitins was attained by tosylation of chitin followed by iodination. They were expected to be useful as precursors for further modifications. Cationic graft copolymerization of styrene onto iodo-chitins resulted in the formation of copolymers with graftings of 300% or more.

(3) Introduction of Functional Groups

Various routes for introduction of new functional groups have been examined. Diethylaminoethylation and some reactions with cyclic compounds were confirmed to have high potentials.

Design of Novel Fanctional Materials Capable of Releasing Biological Information Molecules on Electric Stimulation

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1.Introduction

The biological information system is characterized by molecularcommunication incorporating a variety of information molecules, which is differentiated from the in vitro information system. This investigation aims at the "Molecular Interface" between the biofunctional materials and the biological systems on the basis of molecular communication.

2.Results and Discussion

(1)Electric ally Stimulated Release of Neurotransmitters Modeled on a Presynaptic Membrane has been successfully designed using graphite, polyacetylene and polypyrrole to release neurotransmitters such as acetylcholine and glutamate on electric stimulation. In fiscal year 1988, an improved presynaptic membrane has electrochemically symthesized from pyrrole, which has provided with rapid response to electric stimulation.

(2)Electrochemical Synthesis of Conductive Enzyme Membranes "Conductive Enzyme Membranes" have been synthesized by electrochemical polymerization of pyrrole in the presence of enzymes such as glucose oxidase with retaining enzyme activity and electric conductivity. The novel membrane performed reversible electron transfer via polypyrrole between the enzyme and the metal electrode on which the membrane was depositted. It is noted that enzyme activity is electronically modulated.

Basical studies for the construction of the chimera receptors which mediate new functions

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1. Introduction

Recently, the structures of the receptors have been elucidated by the cDNA cloning technique. The basical structure of the receptors has the extracellular domain which binds to the ligand and the intracellular domain which has a enzyme activity. The purpose of this project is to elucidate the structure and function relationship of the receptors, mainly insulin receptor, in order to construct chimera receptors which have new functions and in order to develop the receptors as biomaterials.

2. Results and Discussion

To test whether the tyrosine kinase activity of the insulin receptor is crucial for insulin action, we have constructed mutations of the human insulin receptor at Lys-1030, which is in the presumed ATP-binding region. By using oligonucleotide-directed mutagenesis, this lysine residue was replaced with either methionine, arginine, or alanine. Chinese hamster ovary cells were transfected by mutant cDNAs and the expressed insulin receptors were characterized. None of these mutants exhibited insulin-activated autophosphorylation and kinase activity in vitro. They also do not mediate insulin-stimulated uptake of 2-deoxyglucose and DNA synthesis. The tyrosine kinase activity is thus required for a key physiolosical response of insulin.

Two chimaeric receptors have been constructed: a chimaeric receptor HIR(α)-IGFIR(β) is comprised of the extracellular domain of the human insulin receptor fused to the transmembrane and cytoplasmic domains of the insulin-like growth factor I receptor. A second chimaeric receptor HIR-IGFIR(tail) is comprised of the extracellular, transmembrane and tyrosine kinase domain of the human insulin receptor fused to the carboxy terminal region of the insulin-like growth factor I receptor.

These chimaeric receptors were expressed in Chinese hamster ovary cells. Although HIR-IGFIR(tail) was incapable of the transmembrane signalling, HIR(α)-IGFIR(β) was capable of it.

Development of Plant Material with Odour Information Transducing Function

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1.Introduction

A promising property of a plant leaf as odour sensig material was formerly demonstrated. Extremely low concentration of odour compounds reversibly modified a characteristic response pattern to 100% CO_2 gas. It is essential to analyze these response mechanisms for the future application of the plant leaf to odour information transducer. The present study has analyzed the response mechanism of a leaf of tobacco (Nicotiana tabacum "Xanthi nc") to 100% CO_2 gas.

2.Results and Discussion

A structure model of a leaf is presented based on the dye diffusion experiments and comparative measurements of cellular potentials at various positions of a tobacco leaf. Characteristic response pattern to 100% CO_2 gas is presented and its profile was divided into ON response (a profile obtained after the initiatiion of CO_2 gas exposure) and OFF response (a profile obtained after the termination of CO_2 gas exposure). A possible participation of H^+ both in ON and OFF responses is speculated. The generation of electrogenic potential is well ascribed to the ATP-dependent proton pump. Thus, using a newly developed sealed reaction vessel, the response to 100% CO_2 gas was investigated without air and light. As the result, the most part of ON response was ascribed to the electrogenic potential. The influence of other ions such as K^+, NH_4^+, and Cl^- was also investigated and a remarkable participation of K^+ in OFF response was demonstrated. Based on these results, the response mechanism of a plant leaf to 100% CO_2 gas and odour compounds is discussed.

Interactions between biological messenger components and polymer materials

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1. Introduction

Polymer materials have been widely used in body as surgical devices and assists of drug delivery, since they are available with a variety of physicochemical properties and can be readily fabricated into many forms. In addition, their surfaces may be modified with ease, but such modifications can exert diverse influence on biological systems.

Since the direct interaction of polymer materials to the systems, composing of many humoral and cellular messenger components, is an inevitable event, it is indispensable to study the interactions between the two to develop biofunctional polymer materials which positively act on the systems. Macrophages (Mϕ) were selected as a representative cell operating as the messenger components. In the present study, Mϕ attachment to polymer films, followed by their interleukin 1 (IL 1) production, was investigated.

2. Results and Discussion

Mϕ attachment to conventional polymer films as well as polyethylene films grafted with polyacrylamide and gelatin was examined. The number of Mϕ attached was plotted against the water contact angle of the films. A distinct dependence of Mϕ attachment on the surface wettability was observed, the optimal wettability for cell attachment being approximately 70° in the water contact angle except for the glass and the gelatin-grafted polyethylene film. This result indicates that the Mϕ attachment to polymer films cannot be explained simply in terms of their surface wettabilities but that van der Waals, Coulombic, and biospecific interactions are concurrently operating between the cells and the films.

IL 1 production by Mϕ, which had attached to the polymer films of different surface wettabilities, was investigated. A maximal activity of IL 1 activity was observed for the films with a water contact angle ranging from 70 to 80°, though extracellular IL 1 activity was low compared with intracellular one. It was reported that both intra- and extracellular IL 1 activities of Mϕ ingesting polystyrene microspheres were enhanced. However, an increase of intracellular activity was observed when attached to polymer films, indicating the dependence of material forms on IL 1 production. Moreover, a close correlation between the intracellular IL 1 activity and the number of Mϕ attached, especially that of Mϕ attached under spreading, was observed.

Condensation of Bioactive Compounds into the Membrane Compartment by Conjugation with Synthetic Polypeptides and Control of the Biological Activity

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1. Introduction

Peptide hormones are shown to take a defined conformation and orientation in lipid membrane. On the basis of this finding, it is postulated that cell membrane should play an important role in the interaction of peptide hormones with receptors. On the other hand, receptors in cell membrane are frequently found to aggregate after binding with peptide hormones, which is also supposed to have an important meaning in the signal transduction. In the present investigation, peptide hormone analogs, which are designed to show a high affinity to lipid membrane or to crosslink the receptors in membrane, were synthesized and investigated on the interaction with the receptor.

2. Results and Discussion

i) Enkephalin analog having a high affinity for lipid membrane and receptor selectivity [Leu]enkephalin was extended to the C terminal with an octapeptide, which takes an amphiphilic helical conformation and shows a high affinity to lipid membrane. The receptor selectivity of this analog was different from that of enkephalin amide, and the affinity for μ receptor subtype came to be comparable to that for δ receptor subtype, indicating that the membrane affinity of the analog affects the selectivity of opioid receptor sybtype.

ii) Bivalent analogs Two enkephalin moleclules were bound together with a spacer between them. The affinity of synthesized bivalent ligands for μ receptor subtype was slightly higher than that of the monovalent ligand. Therefore, the distribution or structure of μ receptors in membrane are suggested to be favorable for the crosslinking by the bivalent ligands.

iii) Polysaccharide conjugated with many enkephalin molecules Several enkephalin molecules were introduced to dextrane. However, the affinity for opioid receptor was lower than that of enkephalin amide, probably due to the steric hindrance of polymer moiety in the interaction with receptor.

Elucidation of Cell-Cell Information Transfer by Using Artificial Cell Liposome and Its Medical Application

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1. Introduction

Saccharide determinants on cell surface are very important in biological recognitions. we have developed new biomaterials such as cellspecific drug carrier or artificial vaccine using the glycolipid, glycoprotein, or polysaccharide derivative-reconstituted liposomes.

2. Results and Discussion

2.1. Development of Artficial Boundary Lipid

First of all, an artificial boundary lipid which bears an amide bond respectively in each acyl-chains of lecithin, 1,2-dimyristoylamido-1,2-deoxyphosphatidylcholine(DDPC), in order for liposome to be more mechanically and biochemically stable and to increase reconstitution efficiency of membrane protein into liposomal membrane.

Several proteins were effectively transfered from human eryhtrocyte to DMPC liposome which contains DDPC. This is very simple and useful methodology to directly reconstitute membrane proteins into liposome.

2.2. Liposomal Vaccine

We have newly developed liposomal vaccines to prevent cancer diseases using ganglioside-reconstituted or tumor surface antigen protein (TSAP)-reconstituted liposome. Of various liposomal vaccines, only a 50 : 1-mixture of G_T(trisialoganglioside) and G_Q(tetrasialoganglioside)-reconstituted liposome showed effective and reasonable rejection against B16 melanoma cells challenged after immunization in C57/6. Interestingly, after vaccination of G_T + G_Q-containing liposome, an antibody of I_gM class for mono(G_M) and di(G_D) sialogangliosides as well as that of G_T was produced. Moreover, antibody of I_gG class for G_M and G_D was also detected in the final stage of vaccination.

TSAP, which was isolated from mice leukemia(Balb RVD), was reconstituted into eggPC-DDPC liposome. Mouse which were immunized by this TSAP-liposome vaccine showed complete rejection of the growth of tumor cells.

DEVELOPMENT OF NEW FUNCTIONAL MATERIALS FOR CONTROL OF DRUG-TRANSPORT ACROSS THE SKIN

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1. INTRODUCTION

In studies of percutaneous absorption, the development of penetration enhancers, to overcome the low permeability of drugs across the skin, is becoming important. In this study, the promoting effect of cyclic monoterpenes present in essential oils on the percutaneous absorption of indomethacin (IMC) was investigated in rats.

2. RESULTS AND DISCUSSION

As compared with Azone, both in vitro permeation coefficient and in vivo absorption of IMC were markedly enhanced by the addition of d-limonene, which is the main component of orange or lemon oils. Similar activity was observed in the cases of the l- and dl-forms of limonene, p-menthane, alpha-terpinene or terpinolene. On the other hand, no effect was obtained, when the additive had hydroxyl or carboxyl groups, or ether oxygen in its chemical structure. Therefore, the lipophilicity of these compounds might play an important role in drug transport across the skin. The effect of pretreatment of skin with d-limonene on subsequent percutaneous absorption of IMC was investigated in order to estimate the influence of d-limonene on the barrier properties of the skin. No effect was obtained suggesting that d-limonene might reversibly alter the skin structure as the barrier for the drug transport. The primary skin irritation of d-limonene examined with rabbit dorsal skin in vivo. The skin irritation of d-limonene was significantly lower than that of Azone. These results suggest that d-limonene offers great promise as an enhancer of absorption in the development of transdermal therapeutic systems.

Synthesis of Monomers Promoting Diffusion into Teeth and Analysis of Hybrid Structure Composed of Cured Monomer and Teeth Component.

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Introduction

In order to bond artificial materials to natiral tissues, new monomers which possss affinity with the tissues were synthesized and dental resin containing the monomers as a functional monomer was prepared. Moreover, bonding interface between the tissues and cured resin was analyzed. In this study, teeth was selected as the tissue.

Results and Discussion

Methacrylates with hydrophobic and hydrophilic groups showed good bonding ability because they are biocompatible and can promote interpenetration of monomers into teeth enamel. The interpenetrated layer on subsurface of teeth enemet formed a hybrid of teeth component and cured resin. Since the fractured surface of teeth enamel showed excellent resistance ability for acid treatment, there was no caries formation when the tooth put into an artificial caries pruducing acid solution. On the other hand, the fractured surface in the case of using conventional dental resin without functional monomers accepted acid etching. This result suggested that the hybrid zone composed of cured resin and teeth component plays an important role for resistance for acid treatment.

Development of Molecular Transport System based on the Alpha-2-macroglobulin:Receptor System

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1. INTRODUCTION

Alpha-2-macroglobulin is one of the most abundant proteins in human plasma. It inhibits many different kinds of proteinases from all four classes by enclosing proteinase molecules within its molecular domain. If proteins other than proteinases are co-present in the solution, some of them become trapped along with the proteinases. The enclosed proteins are effectively protected from the attack of degrading enzymes or specific antibodies in circulation. Since the complex between alpha-2-macroglobulin and proteinase is recognized by a specific receptor on the cell membrane and internalized via receptor mediated endocytosis, this protein has a potential to be used as a vehicle to transport proteins of choice into the cell.

2. RESULTS AND DISCUSSION

First, we studied the stability of proteinases and cytochrome c in the trapped state within alpha-2-macroglobulin. Trypsin and chymotrypsin both became quite stable against self-inactivation during the incubation period at 37°C. Trypsin was not accessible by specific antibodies as checked by ELISA method. When cytochrome c was trapped by alpha-2-macroglobulin by the action of trypsin, it became stable against the attack of external proteinases.

Internalization of radio labelled proteins by cultured cells were studied and the result showed an enhanced transport of such proteins when they were complexed with alpha-2-macroglobulin. The effectiveness of transport showed a saturation curve implying an involvement of receptor mediated endocytosis. The system has a potential to be exploited as a molecular transport system of specific proteins into the cell. A problem to be overcome is intracellular targetting of transported proteins.

Development of Novel Biomimetic Membranes Based on Molecular Recognition and Transport

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1. Introduction.

The aim of this research is to develop solid membranes which exhibit high selectivity, good permeability, and mechanical stability. To fabricate such membranes, we propose surface modification of microporous films or glass membranes with compounds capable of molecular recognition by the Langmuir-Blodgett (LB) or chemical modification techniques. As a first stage toward this end, we have already demonstrated the ion recognizing action of a solid surface functionalized with a crown ether by LB technique. This year we attempted to optimize conditions for surface modification by the LB and chemical modification techniques.

2. Results and discussion.

Multilayers of an amphiphilic crown ether, 6-[bis(tetradecyloxymethyl)methoxyethanoylamino]hexanoylaminomethyl-18-crown-6, was transferred onto an SnO_2 semiconductor electrode by the LB technique. The amphiphile employed here was found to form a stable condensed film through the intermolecular attraction due to hydrogen bonding of amide groups and Van der Waals forces between the long hydrocarbon chains of neighboring molecules.

Diaminodibenzo-18-crown-6 was covalently attached to the SnO_2 surface via (1) 3-aminopropyltriethoxysilane or isopropyltri[N-(2-aminoethyl)-2-aminoethyl]titanate and glutaraldehyde, or (2) butadiene derivative silane coupler and acryloyl chloride.

The surface conductance of the LB film-modified and chemically-modified electrodes was measured under depletion conditions in the aqueous solution of alkali and alkaline earth metal chlorides. The change in surface conductance was greater for the metal ions forming a more stable complex with the crown ether. The complexation changes the depletion layer thickness, and hence the surface conductance of the electrode. The experiments showed that the crown ether on the solid surface recognizes a specific metal cation. The sensitivity of these electrodes was maintained for two months after fabrication.

PREPARATION OF VERY FINE FERRITE PARTICLES BEARING BIOLOGICAL LIGANDS AND THE USE OF THEM IN THE STUDY OF DYNAMIC ENDOCYTOTIC PROCESS.

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Introduction: Almost all the biological cells have specific endocytotic activities of many kinds of biological ligands. This process is a dynamic membrane process in which specific vesicular organelle, called endosomes, play important roles. Isolation and characterization of endosomes shares an important part of the study of endocytotic process. We have developed a new method of studying endocytotic process by preparing very fine ferrite particles coated with biological ligands and use of them in isolation of specific endocytic vesicles by high gradient magnetic separation (HGMS). This study is aimed to provide technological bases of specific, controlled delivery of materials to organs and utilization of specific sorting activities of biological cells.

Results and Discussion: Very fine ferrite particles with the edge length of 5nm were dispersed by coating with oleic acid and further coated with phosphatidylcholine and asialoganglioside. Some acylated marker enzymes such as horse-radish peroxidase (HRP) could also be conjugated. When these ligands were introduced to liver by perfusion, most of them were taken by the parenchymal cells. To isolate endosomes which had taken the ligands, the liver homogenate was applied to the HGMS column. This column contained mesh-works of ferritic stainless wires. When these wires are placed in a magnetic field, very steep magnetic gradient is generated around the wires and this attracts the ferrite-containing endosomes. After washing extensively with buffer, the endosomes were easily recovered by simply turning of the magnet. When the endosomes were isolated after 5 min-perfusion, they contained both ligands and receptors and showed the density of 1.05 in Percoll-density gradient centrifugation. By 15 min-perfusion, we could isolate another population of endosome with the density of 1.08 which lacked receptors but contained the ligands. These endosome should be formed by fission of the early endosomes after sorting of ligands and receptors. The vesicles after 30 min-perfusion peaked at the density higher than 1.12. They were ligand-containing lysosomes sine they showed very high activities of lysosome enzymes. We detected some specific proteins in these different population of endosomes. Characterization of these proteins are under progress and this study would reveal the machinery of sorted membrane process in receptor-mediated endocytosis.

Regulation of Mass Transport Process by Photopolymerized Vesicles Incorporated with Ionophoretic Substances

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1. Introduction

Molecular aggregates were assembled to simulate the regulatory and modulatory mechanisms in biologial cellular systems and to utilize as a functionalized biomaterial. The following artificial cellular aggregates were discussed :

(1) Voltage sensitive vesicles composed of photopolymerizable phospholipids and ionophoretic substances.
(2) Phospholipid inverted micellar aggregates as an ionophore in liquid membrane system.
(3) Temperature sensitive coacervate of α-elastin, a biological elastic fiber-related protein.

2. Results and Discussion

The regulation and modulation of ion transport processes by these molecular aggregates were investigated by electrochemical measurements.

A patch-clamp method was employed to examine a polymerized phospholipid bilayer membrane incorporated with antibiotic ionophores. Incorporations of the channel forming antibiotics into the photopolymerizable lipid bilayer were available as the same manner as into the ordinary lipid bilayer; however, it took an unexpectedly long time to establish the polymerizations.

Ion transport processes across a liquid membrane mediated by the phospholipid inverted micellar aggregates were characterized by the percolation mechanisms. These liquid membrane transport of ions can be regulated by the multiple factors such as the temperature changes and the changes in water contents of membrane fluid.

Coacervation process of α-elastin was affected not only by temeprature raising but also coexisting metal cations. The α-elastin coacervated liquid membrane functioned as a weak acid-weak base type amphoteric ion-exchange membrane. The ion transport phenomena based on a specific interaction of protein molecule with metal cations were also observed.

Development of Stimulus-Responsive Transport Systems

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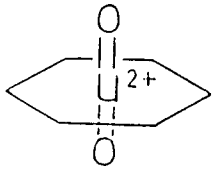
1. Introduction

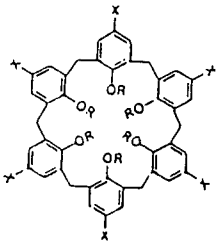
Biological transport systems feature the high selectivity and the controlled transport rate. This aspect may be reconstituted in a totally artificial system by skillfully combining a synthetic receptor with a stimulus-responsive function. With this object in mind, we design transport systems with responsive functions which have within a system both the host molecule serving as a recognition site and the stimulus-responsive group accepting the stimulus from the outside world.

2. Results and Discussion

Hexacarboxylate derivatives of p-t-butylcalix[6]arene (1_6:R = CH_2COOH) extracted uranyl ion (UO_2^{2+}) efficiently and selectively from water to organic media. The high UO_2^{2+} selectivity was attributed to the structure of calix[6]arene properly preorganized for the binding of UO_2^{2+} which requires the pseudo-planar hexadentate coordination. UO_2^{2+} was selectively transported by 1_6 across a liquid membrane from a neutral to an acidic aqueous phase in an active transport manner. The polymer-liquid crystal composite membrane immobilizing 1_6 was prepared: the rate of UO_2^{2+} transport across this thin membrane was efficiently controlled by the changes in pH and temperature. These are novel examples for a stimulus control of selective transport and storage of UO_2^{2+}



pseudo-planar hexadentate
structure of UO_2^{2+} complexes



1_6

Design of Thermosensitive Polymers as On-Off swiches for Drug Release

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1. Introduction

Stimuli-sensitive polymers which change their structure and physical properties in response to external signals have promising potential in the design of drug delively systems. This concept of controlled drug delivery with physical and chemical modulation is proposed. In particular, self-regulating or auto feed-back drug release systems may be achieved by utilizing stimuli-sensitive polymers. Such a system enables drug release in controllable, pulsatile patterns regulated by external modulations. Although the efficacy of pulsatile drug release has not yet been clarified, this new system is of interest in basic and applied fields as a new method to achieve improved drug therapies.

2. Results and Discussion

To improve mechanical properties of thermothensitive hydrogel, isopropylacrylamide (IPAAm)-butyl methacrylate (BMA) crosslinked copolymers were synthesized. Membranes and monolithic devices were prepared using 5% BMA composition of the copolymeric gel which showed both excellent thermosensitivity and mechanical properties. It was confirmed that quick response of the copolymeric gel surface to temperature change was utilized as an on-off switch for drug release and permeation. The data represent the first experimental results showing an on-off swiching phenomena of thermo-sensitive hydrogels which are applicable for modulated pulsaatile-controlled release systems.

Construction of Biomaterials Having Energy-Converting Functions
Development of photocontrollable gel materials and their application

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1. Introduction

It is interesting and important to regulate bioreactions by a certain external signals for the construction of efficient and controllable bioconversion systems. We have found that a semiconductor, titanium (IV) oxide (TiO_2), can catalyze the oxidation of NADH to NAD^+ under illumination of UV light. Entrapment of alcohol dehydrogenase (ADH) and TiO_2 with a certain gel material provided a stable and photocontrollable dehydrogenation system. This report deals with ADH-TiO_2 coupling system with photocatalytic regeneration of NAD^+ in an organic solvent. We also describe the construction of photocontrollable system for glucoamylase (GA) immobilized by entrapment with synthetic polymer gels, in which an artificial photoresponsive substance is incorporated.

2. Results and Discussion

ADH from equine liver (HLADH) was found to catalyze dehydrogenation of cinnamyl alcohol in water-saturated isopropyl ether. Under illumination, HLADH and NAD^+ adsorbed on TiO_2 formed cinnamaldehyde more than 23 times the molar quantity of NAD^+ given initially. The results indicates that photocatalytic regeneration of NAD^+ from NADH and the coupling reaction with HLADH could be successfully carried out in such an HLADH-TiO_2 system in an organic solvent. Condensed localization of HLADH and NAD^+ on TiO_2 particles in the organic solvent system provides an effective coupling reaction compared with an aqueous system where it is necessary to use a high concentration of NAD^+.

GA entrapped with long chain synthetic prepolymers showed higher activity than that with shorter ones with starch as substrate, although the activity was low compared with the free enzyme. This relationship between gel network and enzyme activity on substrate diffusion and apparent enzyme activity is useful to design a photocontrollable biomaterial system with photoresponsive polymers.

Azobenzenes undergo isomerization from trans form to cis form under UV light with a large structural change, and the reverse is observed with visible light. We have attemted to incorporate azobenzene residues as the pendant groups into polymer gels entrapping GA. Although azobenzene residues in polyurethane gels showed good reversible photoisomerization, the activity of GA in the gels could not be controlled by UV light-visible light. We are now trying to introduce azobenzene residues into the backbone of the polymer gels expecting a large conformation change to be induced by light.

Construction of a photo-controllable enzyme reaction system by co-immobilization of an enzyme and a semiconductor

Akihisa Aoki, Mitsuyoshi Ueda, Hiroki Nakajima and Atsuo Tanaka

Biocatalysis, in press (1988)

Novel photocatalytic NAD^+ recycling system with semiconductor in organic solvent

Takuo Kawamoto, Akihisa Aoki, Kenji Sonomoto and Atsuo Tanaka

J. Ferment. Bioeng., in press (1989)

Study of Artificial Muscle

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1. Introduction

If we will apply the polymer gels to a mechanochemical system as an artificial muscle, we must first consider the response time and the mechanical properties, such as modulus and strength, as well as the swelling ratio. In order to get an artificial muscle acting just like a human muscle from polymeric materials, we have to pursue a plan to get the values of contraction power of 6～10kg/cm^2, a response time as high as a second order and a sensitive responsibility to electric stimulation.

2. Results and Discussion

In order to introduce the crosslink structure and ionic groups into poly(acrylonitrile) (PAN) fibers, the PAN fibers were annealed at 220℃ in air for ca.5 hours (preoxidation process) and subsequently boiled in 1N.NaOH aqueous solution for 30 min (hydrolysis process). The fibers show a typical elongation and contraction behavior when the gel fibers was immersed in 1N.NaOH solution from 1N.HCl (elongation) or vice versa (contraction) in an isotonic state under 0.75 kg/cm^2 load. The swelling ratio was 60～80%, the contraction speed was less than 2 seconds and the contraction force showed ca.20 kg/cm^2. Such values of elongation and contraction behavior are similar in magnitude to a human muscle. According to the equilibrium gel length in isotonic state as a function of the pH value, when the pH value was decreased from 14 to 0, the swollen gel fibers was contracted at pH 3. On the other hand, the gel fibers was elongated at pH 11 when the pH was increased from 0 to 14. Such hysteresis curve in the elongation/contraction behavior could be related to the interaction between molecules in the gel network, such as formation of hydrogen bonding between carboxylic groups and pyridine rings. As an increase in the ionic strength controlled by NaCl solution, the hysteresis loop was changed and finally disappeared. That is, the gel fibers showed the abrupt change only at pH 3 under the ionic strength of 1. Also, the PAN gel fibers can be contracted and elongated by changes in concentration of calcium and sodium ions.

Design of New Material Containing Bio-functional Elements with Controlled Super Structures

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1.Introduction

To design models and utilize the high functions of biological membranes such as highly efficient energy conversion and specific responses we must fully consider their structural asymmetry or anisotropy which constitute a major basis of their functionalities.

Our purpose is on the artificial control of supra-higher order structure of the bio-membrane elements in synthetic materials.

2.Results and Discussion

As the first attempt, continuing from the last year, we have prepared asymmetric functional membranes by using a static electric field. This method is based on the ionic nature of co-monomer or enzyme proteins responsive to a given electric field and influence the distribution or orientation of the protein by applying an external electric field.

Bacteriorhodopsin (BRp), found in purple membranes (PM) of cell membranes of Halobacterium and known for its proton translocating property upon light illumination. The orientation of BRp in the polymer matrix was efficiently influenced by application of external electric field. In this year efforts were made mainly on further improvement of controlled orientation using direct filed application by use of pulse method or by multi-phase matrix which is consist of two or three layers of polymer matrices of successive polymerization sandwiching oriented thin PM layer.

The second target was set on the membrane bound oxido-reductase such as cytchrome C oxidase, the orientation of which is also crucial to the energy conversion steps in the mitchondrial membrane system. Rather fundamental studies for this target were performed during this year and the electrode processes containing soluble and globular model oxide-reductase such as glucose oxidase and diaphorase(both of which are flavo-enzymes) were investigated. Electron delivery between the Au-plated polyethylene cloth and the flavo-protein in protein-immobilized systems was investigated and successful results were obtained.

Bioelectronic Materials Based on Peptides and Proteins Containing Functionalized Artificial Amino Acids

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1. Introduction

Bioelectronic materials, in this study, may be defined as proteins and synthetic polypeptides in which the transport of one electron can be regulated by light or electric field. In order to provide the artificial function in the proteins or polypeptides, the synthesis of artificial amino acids carrying photo- or electro-functional side chains and the incorporation of these artificial amino acids to proteins or polypeptides were attempted.

2. Results and Discussion

(1) A Very Fast Excitation Energy Migration along a One-Dimensional Array of Anthryl Chromophores

In a sequential polypeptide having a repeating unit of [Lys(Z)-Lys(Z)-9-antAla] has been synthesized. The risetime of the excimer emission of this polypeptides was shorter than 100 ps, indicating that the photoenergy absorbed by an anthryl group migrates very rapidly along the chromophoric array.

(2) First Measurement of the Electron Transfer Rates along a Helical Polypeptide Chain

The rate of electron transfer from a p-dimethylanilide group (D) to a pyrenyl group (P), both being fixed on the midway of a helical polypeptide chain, was first measured. The rate was in the order of 10^7 when the D-P pair is separated by 5.5 Å and in the order of 10^5 Å when 9.3 Å. The electron transfer rate was nearly temperature independent over the range of 20°C - -196°C.

(3) Photoregulated Aggregation of Azobenzene-Containing Peptide-Phospholipid

A tetrapeptide carrying an azobenzene group was covalently linked to diphosphatidylethanolamine. The peptide-phospholipid in water showed a reversible change between solution and aggregation, induced by photo-irradiation.

Photostimulated Shape Change of Synthetic Polymers

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1. Introduction

Biological systems have developed various kinds of photoactive organs to adopt themselves to sunlight. In fungi, for example, a phototropism system has been evolved to find favorable conditions for survival. Although, it is impossible to reconstruct the biological organ itself, it is worthwhile to use the principle of the photoresponsiveness for molecular design of artificial photoresponsive systems. The purpose of the present study is to construct an artificial photoresponsive polymer, which changes the shape in response to the light.

2. Results and Discussion

If we intend to make a sensitive photoresponsive polymer gels, which change the shape efficiently in response to a small number of photons, it is necessary to introduce an amplification mechanism to the system. A convenient way is to utilize a phase transition of polymer systems. As a model of the photostimulated phase transition, we examined the phase separation of an aqueous solution of poly(N-isopropylacrylamide) with pendant azobenzene groups. Before photoirradiation, the 1% aqueous solution of the polymer became turbid upon heating and the transmittance decreased to one-half the initial value at 19.4°C. Upon ultraviolet irradiation($410\ nm > \lambda > 350\ nm$), the phase separation temperature increased to 26.0°C. The phase separation temperature depends on subtle balance between the hydrogen bond formation ability of the polymer with water and intermolecular hydrophobic force. A small number of azobenzene chromophores(2 - 3 mol%) affect the balance and the phase separation is induced efficiently. Although the idea was extended to a polymer gel system, the amplification effect so far observed was small in the gel.

An electric field effect on the photostimulated gel bending motion was also examined. Under an electric field 10 V/cm, the gel bent in less than 2 min. By the addition of salts, such as NaCl or $MgCl_2$, the bending rate was accelerated. Under an alternate electric field, the gel showed a vibrational motion.

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Multiple Effect Manifestation Function

Multiple Effect in Cell-Material Interactions

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1. Introduction

As the number of liver failure increases, the expectation for hybrid artificial liver, hapatic biosimulator for drug design and hepatocyte container for liver bank is getting higher and higher. The need for efficient separation of hepatocytes (parenchymal cells) is also significant. The most important problem is to design good substrata for hepatocytes culture.

2. Results and Discussion

Some effects of sodium Benzoate (BA-Na) on adherent cell shape and expression of cellular functions of cultured hepatocytes on collagen and PVLA substrata were examined using adult rat hepatocytes in primary culture. The cell-spreading activity of cultured hepatocytes on collagen substratum was clearly suppressed in the medium supplemented with BA-Na, whereas they usually exhibited the cell-spreading activity on collagen substratum under the nomal conditions. Furthermore, multilayer aggregation of hepatocytes on PVLA substratum was clearly suppressed in the presence of BA-Na, too. On the contrary, albumin and bile acid synthesis activity were enhanced as the concentration of BA-Na increased up. Especially, the behavior of bile acid synthesis activity prominently increased. But, it was observed that the leakage of GOT and GPT were decreased as the concentration of BA-Na increased up. On the other hand, the amount of intracellular DNA decreased dose-dependently.

The cell culture system used here has potential advantages that we can explicate the relation between adherent cell shape and expression of hepatocyte-specific functions, and the reciprocal relation between cell growth and expression of hepatocyte-specific function.

Molecular Design of Polymer Surfaces Which Acivate Humoral and Cellular Body Defense Mechanisms and Development of "Immuno-Activator"

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1.Introduction:

This research aims to establish the molecular design criteria of design of polymer surfaces on which major body defense mechanisms are selectively and potently activated, and to develop an immunoactivator", an extracorporeal device, which is capable of enhancing the immune system upon extracorporeal circulation. The first-year of the research project focused on surface design by chemical modification of polymer surface functional groups present at the outermostlayer. In the second year, much efforts have been paid to quantify the surface density and its depth profile.

2.Results and Discussion

In order to quantitatively assess the surface density of introduced functional groups and its depth profile, theoretical formulation on analyzing the depth profiling was materialized, which was based on the diffusion control model. Measured ESCA data fitted very well the theoretical treatments. The conclusion withdrawn was summarized as follow; irrespective of the solvents used, the surface chemical modifications of carboxylation and isocyanation proceed via diffusion control.

The chemical fixation of proteins via hydrophilic spacer was performed with the couplers which was prepared in the first year of the project.

Analysis of various effects appeared in the combination use of biomedical materials and cells which compose the blood vessel wall

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I. Introduction

Our previous study revealed that an ultra-fine polyester fibers (UFPF) has a special character to enhance the fibroblasts migration and proliferation in its matrix. These migrating fibroblasts require nutrition. Therefore, capillary blood vessels may be followed by the migration. By this theory, angiogenesis in the vascular graft made of UFPF may be observed. In this study, the angiogenesis of the graft was analysed.

2. Materials and methods

A mesh tube made of UFPF (thickness:3 um), 3 mm in internal diameter, was used. A silicone rubber was introduced into the mesh tube and this combination was introduced into the subcutaneous layer of an experimental animal. After 3 weeks implantation, the tube was removed with surrounded connective tissue, the silicone rod was withdrawn. Then it was implanted into the both carotid arteries of the same animal.

3. Results and discussion.

The connective tissue tube obtained from the subcutaneous layer contained a great amounts of fibroblasts and numerous capillary blood vessels. After the implantation as the vascular graft, it showed rapid endothelialization with the numerous colonies formation of endothelial cells which have openings of capillary blood vessels in each colony. The graft showed perfect thromboresistance because of endothelialization with its natural antithrombogenicity.

From these results, it was concluded that the UFPF enhanced the fibroblasts migration which required the nutrition, and capillary blood vessels were induced. Endothelialization was followed by the colony formation by the opening of the blood vessels in the graft surface. These angiogenesis was considered to be appeared by the combination work of the UFPF and fibroblasts migrated into the UFPF matrix.

Composite Polymer Materials with Proteolysis-Controlling Function

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1. Introduction

The purpose of this study is to design composite polymer materials having a function of controlling proteolysis that such materials may encounter when they are used in vivo. When polymer materials are enbedded into human (or any other animal) tissues, these materials become a subject of the attack by various cellular proteases, of which calpain is one potent example. Two different approaches for designing inhibitory materials were investigated.

2. Results and Discussion

Several analogues of leupeptin (Ac-Leu-Leu-Arg-al) were synthesized, which included phenylbutanoyl-Leu-Met-al, CZ-Leu-Nle-al, etc. These peptidyl aldehyde derivatives were found to inhibit strongly calpains I and II, but most of the analogues inhibited also cathepsins L and B. Attempts to obtain a synthetic inhibitor strictly specific for calpains have not been successful.

Calpastatin is a naturally occurring inhibitor known to be strictly specific for calpain. The elucidation of four times internally repetitive domain structure of calpastatin molecule enabled us to study the inhibitory function of a unit domain and the shorter fragments thereof. Thus, the central consensus sequence of approximately 20 amino acid residues among four domains was elucidated. The proper method for the immobilization of such peptide fragments to design functioning biomaterials is to be established by future studies.

Control of growth and adhesion of primary cultured hepatocyte by polymer substrates

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1.Intruduction

Hepatocytes do not live by themselves, but only under the control of hormons, neighboring hepatocytes and other cells and biomatrix, they are grown and cultured. Especially in early stage of the culture, cell attaching onto well mediated substrate is preliminaly important. The purpose of this study is to design and prepare the polymer surfaces which can control the attaching and growth of primary cultured hepatocytes with synthetic materials.

2.result and discussion

Five kinds of copolymers containing styrene and aminomethyl styrene in various component ratios (AM-10,30,50,70,90 number suggested aminomethyl styrene contents in feed) were prepared by radical polymerization in solution. They are casted on the glass plates from acetic acid containing solution. The wetting and eletrostatic characteristics on their surfaces were investigated by measuring surface tension and Xray photon spectroscopy. The values of nondispersion and dispersion force contribution of solid-water interfacial surface tension (r_{sv}^{p},r_{sv}^{d}) on these copolymer films suggested the formation of specific surfaces on AM-10 and AM-90, against the anticipation that the values of these parameter changed according to the composition of AMSt in copolymers. AM-10 surface showed most hydrophilic and AM-90 most hydrophobic. The contents of free amino group at the outermostly layer in copolymers, investigated by XPS measurments, increased proportionally with AMSt composition. Canine hepatocites were cultured on surfaces of these copolymers by way of the system reported last year. AM-90 surface showed excellent properties on cell attachment, growth, and urenogenesis. These functions were identical to those found in the primary monolayer cultured hepatosytes on collagen coated dishes by addition of FCS. Thus the free amino groups sorounded in hydrophobic environment formed at the outermostly layer of AM-90 enhanced the cell functions in high level.

Development of Man-Made Systems Endowed with Perception

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1. Introduction

We do not aim at making a sensor which simply detects external information sensitively, but at developing an artificial membrane system which is capable of perceiving and judging just like the "brain". At present, however, we do not know the principle of how to design such a system artificially. A single living cell is a chemical system, and yet can sense and judge external stimuli and behave accordingly. So we have studied the mechanism of information processing in amoeboid cell behavior as the first step forward.

2. Results and Discussion

Spatio-temporal patterns of intracellular chemicals are studied in relation to phototaxis by the slime mold. Superoxides are generated upon irradiation, and its action spectrum agrees with that of photobehavior. So superoxides are not only poisons, but also seem to function as a signaling substance. Soon afterwards, intracellular concentrations of chemicals such as cAMP, cGMP, ATP and NADH vary oscillatorily, and eventually different spatial ATP patterns are established. These self-organized chemical patterns may affect the organization of cytoskeletons, and hence a cell behavior.

Amoeboid cells are oscillatory, and hence may be considered as a collection of 2D oscillators. Collective behaviors in this system are analyzed with use of microcomputer image processing. Phase gradient vectors orient pointing toward and away from local stimulation with attractants and repellents, respectively. Inversely, by oscillating the external stimulation, we can modify the orientation of phase gradient vectors and tactic behaviors as well. When stimulated with stationary high temperature, the phase at the stimulated region advances and positive taxis takes place. When oscillated slightly slower than the intrinsic rhythm, however, the phase at the stimulated region becomes retarded and the taxis turns into negative.

Thus, phase relationship among non-linear oscillators, possibly chemical oscillations in non-equilibrium system, is used for information transmission and processing in the living cell.

Immobilization of Enzymes and Their Application to Synthesis of Useful Substances in Organic Solvents

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1. Introduction

Recently, much attention has been focused on enzymatic reactions in organic solvents as novel methods of the preparation of many useful substances. Typical examples are peptide or ester synthesis and optical resolution by proteases or lipases. Enzymes are liable to deactivation by direct contact with orgnaic solvents, and therefore immobilization of enzymes is important for not only recycle usage but also maintenance of catalytic activity. The present study aims at search for effective support materials for enzyme immobilization and characterization of the immobilized enzymes as synthetic catalysts in organic solvents.

2. Results and Discussion

Since the hydrolysis of esters or peptides are reversible, proteases can be catalysts for synthesis of theses compounds in organic solvents containing small amounts of water. A number of support materials were examined for immobilization of α-chymotrypsin (CT), subtilisin BPN' (nagarse) (STB), and subtilisin Carlsberg (STC), and the followings were found to be effective:

1) Enzyme/phosphate salt complexes: In ethanol and other hydrophilic organic solvents, solid CT/phosphate salt complexes are stable catalysts for peptide or ester formation from aromatic amino acid derivatives by condensation or substitution reactions. The reactions are strictly selective for L-aromatic amino acids, indicating that the native conformation of CT is maintained in these reaction media.

2) PVA-immobilized enzymes: Catalytic activity of CT and STB for ester or peptide synthesis increased markedly by immobilization to membrane or gellous PVA. In PVA matrix, enzymes are considered fully hydrated, and detachment is negligible without covalent binding to PVA.

3) Enzyme/chitin or chitosan complexes: The apparent activity of enzymes in organic solvents are surprizingly enhanced by addition of chitin or chitosan. This may be attributed to specific interaction between enzymes and these polysaccharides, since aminoethyl cellulose is ineffective. Crosslinked chitosan beads are also effective supports through physical or ionic adsorption of enzymes.

THE BASIC STUDY ON THE DESIGN CRITERIA OF A BLOOD COMPATIBLE MATERIAL FOR ARTIFICIAL ORGANS

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1. Introduction

The objectives of this study are to develop a new evaluation method of long-term blood compatibility to clarify its mechanism, and to apply the materials evaluated their superiority in blood compatibility to artificial organs.

2. Results and Discussion

The following results have been obtained in this year.

1)Comparison of blood compatibility of segmented polyurethanes between in-vitro and in-vivo experimental results

Seven kinds of segmented polyurethanes were tested in this study. As the results of in-vitro experiment, 1)the outermost surface N-content enhanced the hole blood coagulation, contact activation of coagulation system and complement activation, 2)the increase in contact activation of coagulation system correlated with the shortening of whole blood coagulation time, 3)KP-13 which is segmented polyurethane containing block copolymer of polydimethylsiloxane and polyethylene glycol as the soft segment, revealed the most superior blood compatibility. There was nocorrelation between in-vitro and in-vivo results. However, KP-13 also revealed the most superior blood compatibility in in-vivo experiment.

2)Application of KP-13 to the artificial heart(AH) pump

The inner surface of an AH pump made of PVC was coated by KP-13. The pump was connected to goats as the total AH(TAH) and the left heart bypass(LHB) that survived for 104 days and 49 days, respectively. No thrombus was found inside the pump including around the valve, as well as at the each organs in TAH experiment. The macroscopic thrombus was formed at a part of the sac and around the valve in the low flow LHB experiment, however which was slight comparing with the Cardiothane coated pump.

3)Development of a new AH valve made by a polymer membrane

A new AH valve named jellyfish valve, was developed in which a thin Cardiothane or KP-13 membrane was fixed at its center. The valve was installed into the bloodpump and tested its performance, durability and blood compatibility in in-vitro and in-vivo experiments. The valve revealed the excellent performance which was same with Bjork-Shiley valve, and was durable for more than 4 months. No thrombus was formed on the polymer membrane. Although slight thrombus was formed at the clearance between valve seat and pump nozzle, it would be protectable by gluening both without imperfection.

Development of Biofuctional Membrane with Cascade Amplification and its Applications

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Enzyme cascade system would be useful to enhance the sensitivery. Blood coagulation system of Limulus Amebocyte Lysate (LAL) is possible to determine picogram of lipopolysaccaride (LPS). LPS is considered to be substituted for radioisotopes, enzymes and fluorescent immunoassay. Human IgG(hIgG) was labelled with LPS using covalently binding method as follow. The sugar moietyies of LPS were oxidised by $NaIO_4$ for 1 hr. After desalting, IgG was mixed with oxidized LPS for 2hr. The reaction mixture was cooled in ice. water-and $NaBH_4$ solution. LPS-hIgG conjugate was purified by gelchromatography. hIgG was immobilized on glass beads. LPS and LPS-IgG were quantificated by the measurement of coagulation time of LAL using Toxinometer. The gelation of LAL was also measured by quartz crystal. Resonant frequency of quartz crystal changes according to peocess of the gelation. Admittance of the electrical equivalent circuit of quartz crystal was caluculated using a impedance analyzer and a micro computer. Detection limit of LPS was 1 pg/ml in this system.

hIgG - LPS was used for competitive immunoassay using polyclonal anti-human IgG bound to a polystylene bead as a capture. After immunobinding step, the supernatent was applied to the LAL test, and the coagulation time was measured. Using this method, we were able to measure the presence of hIgG in the range of 10pg/ml $\sim$ 10ng/ml. hIgG was detectable at concentration 1$\sim$2 order of magnitude than previous enzyme immunoassay.

Structurally Controlled Synthesis of Polysaccharides and Their Molecular Biofunctions

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1. Introduction

The present research aims at developing new polymeric materials with molecular biofunctions. They are based on the structurally controlled synthesis of polysaccharides from both carbohydrate and non-carbohydrate sources. Two main subjects are included: 1) Synthesis and functions of amphiphilic block copolymers containing polysaccharides as one of their components. 2) Synthesis of oligosaccharide-carrying polymers and branched polysaccharides, and their application to cell culture.

2. Results and Discussion

(1) An amphiphilic block copolymer consisting of hydrophilic (1→6)-α-D-glucan (dextran) and hydrophobic (1→6)-α-linked polysaccharide skeleton segments was synthesized by the ring-opening polymerization of 6,8-dioxabicyclo[3.2.1]octane with a polysaccharide macroinitiator having a reactive chlorine atom on the anomeric carbon at the reducing end.

(2) Three (1→6)-β-linked polysaccharides were successfully prepared by cationic ring-opening polymerization of 1,6-anhydrosugar derivatives: (a) 4-deoxy-(1→6)-β-L- and DL-ribohexopyranans via propagation accompanied by oxonium exchange reaction, and (b) (1→6)-β-D-galactopyranan by neighboring group participation of a 2(a)-benzoate group.

(3) Oligosaccharide-carrying polymers were synthesized from cellobiose and melibiose as starting materials via radical homopolymerization of styrene derivatives. The cellobiose-carrying polystyrene was found to be usefull as substrates of adhesion of blood-wall cells. The melibiose-carrying polystyrene interacted with red blood cells and aggregated them effectively.

(4) Ring-opening homopolymerization of anhydro-disaccharide derivatives was carried out to give a comb-shaped branched polysaccharide having a galactose moiety in each repeating unit. A polypeptide having β-D-galactose as recognition markers have been prepared via polymeric reactions onto commericial polypeptide.

Design and Preparation of Multi-functional Enzymes with High Thermostability, and Control of Their Functions

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1. Introduction

A variety of enzymes are widely used nowadays as "bio-catalysts" in organic chemistry. However, their physical instability as proteins and the high specificities for substrate structures and reactions are often disadvantageous for further utilization as catalysts in chemical industries. The present study has been undertaken (1) to discover useful enzymes with high thermostability from natural sources, (2) to design and develop new enzymes which show "super-thermostability" by chemical modification and protein engineering, (3) to convert enzymes catalyzing a single reaction into those that can catalyze several reactions under different conditions, and (4) to establish the method for controlling the multi-functions.

2. Results and Discussion

$NAD(P)^+$-dependent amino acid dehydrogenases catalyze the oxidative deamination of amino acids into keto acids and ammonia reversibly. Among them, leucine dehydrogenase is very useful as an anti-tumor drug, a clinical diagnostic agent, and a catalyst in the synthesis of L-branched-chain amino acids in an industrial scale. We have found the occurrence of the thermostable enzymes in cell extracts of *Bacillus stearothermophilus* and *Clostridium thermoaceticum*, purified them to homogeneity, and characterized enzymologically. We also cloned the genes for the thermostable leucine dehydrogenases into *Escherichia coli*. The high expression of the cloned genes in *E. coli* facilitated us to develop a very efficient method of large-scale purification of the enzymes.

The nucleotide sequence including the 1287 base pair coding region of the leucine dehydrogenase gene of *B. stearothermophilus* was determined by the dideoxy chain termination method. The translated amino acid sequence contained 429 amino acid residues corresponding to the subunit (M_r 49,000) of the hexameric enzyme. Comparison of the amino acid sequence of leucine dehydrogenase with those of other pyridine nucleotide dependent oxidoreductases registered in a protein data bank revealed significant sequence similarity in the regions containing the coenzyme binding domain and certain specific residues with catalytic importance. On the basis of the primary structure established, new leucine dehydrogenases with "super-thermostability" and "multi-functionality" have been designed by the use of techniques of site-directed mutagenesis.

Design of Bioactive Materials for Artificial Bone

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1. Introduction

A glass-ceramic containing crystalline apatite and wollastonite in a MgO-CaO-SiO_2 glassy matrix bonds to living bone in a short period, whereas that containing the same kinds of crystalline phases in the glassy matrix added with Al_2O_3 does not bond. The purpose of this research is to reveal principles for designing bioactive materials useful for repairing bone defects by investigating the reason for the difference in their bioactivities. As to this problem, the present author already showed that the essential condition for a glass-ceramic to bond to living bone is to form an apatite layer on its surface in a body environment, and that the apatite layer is formed by a chemical reaction of some elements dissolved from the glass-ceramic with the surrounding body fluid. In this study, what kind of elements are important for formation of the apatite layer was investigated.

2. Results and Discussion

The Al_2O_3-containing non-bioactive glass-ceramic described above did not form the apatite layer on its surface even in the simulated body fluid added with Ca, P, Si or F ion alone, as in the original fluid, but formed in the fluid added with Ca in combination with Si. This indicates that dissolution of the Ca in combination with the and Si from the glass-ceramic is important for formation of the apatite layer on the surface of glass-ceramic. This was confirmed from the result that P_2O_5-free $CaO{\cdot}SiO_2$ glass also formed the apatite layer on its surface in the simulated body fluid. The Ca might increase the degree of the supersatulation of the surrounding body fluid with respect to the apatite while the Si might provide favorable sites for nucleation of the apatite on the surface of the glass-ceramic. This result indicates possibilities that various kinds of bioactive materials are obtained from the system based on CaO-SiO_2.

Novel Medical and Biotechnological Materials from Chitin and Chitosan

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1. Introduction

Chitin is a (1->4)-linked 2-acetamido-2-deoxy-beta-D-glucan and chitosan is N-deacetylated chitin. These biopolymers are naturally abundant. The aim of the present study is to design and to prepare novel biocompatible and functional materials from these biopolymers, and to control their functionality.

2. Results and Discussion

1) Control of the hydrolysis rate of chitin by lysozyme.

We found that the hydrolysis rate of chitin by lysozyme can be controlled by the chemical modification of chitin, indicating that chitin can be usable as a digestible support for drugs at different rate in drug delivery system. We examined the effects of the structure of N-acyl groups of chitin and their degree of substitution on the hydrolysis rate by lysozyme *in vitro*. The hydrolysis rate decreased by replacing H at alpha and beta positions of N-acyl group with NH_2, OH, CH_3, but was completely inhibited by replacing with SH and halogens. The hydrolysis rate also increased by partial N-deacetylation at d.s. 0.5-0.8 and by oxidizing nonreducing end group with periodate. These data indicate that lysozyme digests not only chitin but also its derivatives, because of a broad substrate specificity of the enzyme. We can use the enzyme specificity for a novel drug delivery system.

2) Chitosan for oral administration in drug delivery system.

We found that chitin and chitosan can be usable as a digestible support for the oral administration of drugs in several animals. Chitin and chitosan were digested at 75-95% digestibility by egg-laying hens, broilers, and rabbits, but N-stearoylchitosan was not digested. These data indicate that chitin and chitosan can be usable as carriers for the oral administration of drugs to specific digestive organs.

Synthesis of biofunctional fine-materials.

Biointeraction and clinical application of synthetic materials.

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1. Introduction and Materials used.

The aim of our study was to clarify biocompatibility of glass ceramics and adhesion mechanism of culture cells to glass ceramics. Further their effects on calcification was examined.

Four established culture cell lines, human fibrosarcoma cells (HT-1080), human gingival carcinoma cells (Ca9-22), human osteosarcoma cells (NY) and mouse osteoblast (MC3T3-E1) were used. For phase-contrast and electron microscopic observation they were cultured on substrates of glass ceramics or polystyrene cover srips as control. In addition NY and MC3T3-E1 were cultured with granules of glass ceramics and these cell's intracytoplasmic calcification was observed.

2. Results and Discussion.

Glass ceramics brought neither cellular degeneration or death on phase contrast microscopy. On transmission electron microscopy amorphous structure similar to basal lamina was observed on interface between substrates and Ca9-22 and NY. Similar structure existed between glass ceramics and MC3T3-E1. HT-1080 showed no such structure. Calcification of NY and MC3T3-E1 were increased on phase-contrast microscopy with Von Kossa and Alizarin Red-S staining.

These findings suggested that biocompatibility of glass ceramics was satisfactory. Further from clinical point of view it seemed to be possible to close material-tissue interface with epithelial, fibrocytic and osteocytic cells and to accelerate ossification around this material.

Structure and Functionality Relationship of Oligopeptide-Immobilized Bio-Adhesive Materials

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1. Introduction

Along with the structural elucidation on glucoproteins carrying cell-adhesion and cell-multiplication activities, such as fibronectin and chondronectin, specific oligopeptides Arg-Gly-Asp-X, where X denotes an amino acid residue, are suggested to participate in the cell-attachment activity.

The purpose of this work is to synthesize the oligopeptides including Ser, Val, Ala or Thr as the X component, and their sequential polymers, immobilize them on amphipatic polymer materials, analyse the surface properties of the materials, and then investigate the interactions of the materials with various kinds of cells.

2. Results and Discussion

An oligopeptide, Arg-Gly-Asp-Ser, was synthesized by solid phase method (improved Merrifield method) as well as liquid phase method. Large scale synthesis of the tetra-peptide was carried out by the latter method, in which purity of the product was confirmed by thin layer chromatography step by step. Elimination of the protective residues at the final step was performed by TFMSA. The composition of the final product was confirmed by aminoacid analysis. Oligo-glycin, $(Gly)_n$, where $n = 1 \sim 3$, was used as a spacer by connecting it at N- or C-terminal of Arg-Gly-Asp-Ser. So far, the tetrapeptide was immobilized on PVA and on PAA, an ethylene-acrylic acid copolymer, in the presence or absence of $(Gly)_3$. Surface characterzation and adsorption of plasma proteins on these samples were already carried out.

With polyvinyl alcohol film immobilized Arg-Gly-Asp-Ser at its N-terminal without spacer, cell-attachment tests were carried out by using fibroblast cell (L-cell) originating in Epitherial cell (mouse). It was pointed out that the cell attachments at incubation time 5 hrs were 30- and 10-fold of the contrasts, respectively, in the presence and absence of serum.

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